REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-02-

0081

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	21 Feb 02	Final Report 01 Jul 98 to 31 Aug 01

4. TITLE AND SUBTITLE
DEPSCOR/97-98 MECHANISMS AND BIOMONITORING OF TOXICANT-INDUCED CHANGES IN ZINC FINGER PROTEINS

5. FUNDING NUMBERS
F49620-98-0486
98-10486
3484/BS
61103D

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9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
801 N Randolph Street
Arlington VA 22203-1977

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Human disease result from alterations in biochemical and genetic processes as well as from perturbations induced in these processes by toxic chemicals from the environment. The goals of this project were to understand alterations in gene expression mechanisms induced by toxic chemicals and to develop biomonitoring assays for such toxicants helpful for risk assessment. Inhibitory mechanisms of toxic chemicals on gene expression in vitro: The toxicants lead, mercury, and chromium were found to inhibit protein-DNA interations involved in gene regulatory events by binding to the DNA binding domains of the proteins. Inhibitory mechanisms of toxic chemicals on gene expression in vivo: DNA array and proteomic studies were initiated on organis from rats exposed to lead ions or JP-8 jet fuel. Lead ions up-regulated oncogenes possible accounting for the carcinogenic potential of this compound. Significantly, JP-8 down-regulated a large number of rat intestinal genes and up-regulated genes in exposed lung tissues.

20020322 053

14. SUBJECT TERMS			15. NUMBER OF PAGES
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

Final

Summary/Progress Report, Airforce Office of Scientific Research

Period covered.....July 1, 1998 to August 31, 2001
Title of proposal.....Mechanisms and biomonitoring of toxicant-induced changes in zinc finger proteins.
Grant no..... DAAG55-97-R-BAA3
Name of Institution..... University of Oklahoma College of Medicine
Principal investigator.....Jay S. Hanas, Ph.D., Associate Professor

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Project Summary: Human diseases result from alterations in biochemical and genetic processes as well as from perturbations induced in these processes by toxic chemicals from the environment. The goals of this project were to understand alterations in gene expression mechanisms induced by toxic chemicals and to develop biomonitoring assays for such toxicants helpful for risk assessment. Inhibitory mechanisms of toxic chemicals on gene expression *in vitro*: The toxicants lead, mercury, and chromium were found to inhibit protein-DNA interactions involved in gene regulatory events by binding to the DNA binding domains of the proteins. Inhibitory mechanisms of toxic chemicals on gene expression *in vivo*: DNA array and proteomic studies were initiated on organs from rats exposed to lead ions or JP-8 jet fuel. Lead ions up-regulated oncogenes possibly accounting for the carcinogenic potential of this compound. Significantly, JP-8 down-regulated a large number of rat intestinal genes and up-regulated genes in exposed lung tissues. Development of mass spectrometry to analyze toxicant effects on biological processes: Funds were used to purchase a ThermoFinnigan LCQ mass spectrometer which is capable of sequencing proteins via tandem MS/MS. This technology will be utilized in proteomic analysis of toxicant alterations of gene expression and will complement the electrospray-ionization time-of-flight mass spectrometer already used in the laboratory to analyze ligand-polypeptide interactions. ZifTox biomonitoring assay: The ZifTox assay was developed to assay for a broad range of toxicants in water and soil samples. It depends upon the principal that the toxicants will inhibit binding of zinc fingers to DNA. This assay successfully identified polluted water samples from toxic sites at Tinker AirForce Base in Midwest City, OK. Personnel training, collaborations, publications, patents: Three technicians, two post-docs, and four students were trained in molecular toxicology. Six collaborations, seven publications, 4 manuscripts, and 1 patent (U.S. Patent No. 6,235,538; serial no. 09/034,705) resulted from this project.

WJC

Final ~~Progress~~ and Transition Report, Airforce Office of Scientific Research

Period covered.....September 1, 2000 to August 31, 2001

Title of proposal.....Mechanisms and biomonitoring of toxicant-induced changes in zinc finger proteins.

Grant no.....DAAG55-97-R-BAA3

Name of Institution.....University of Oklahoma College of Medicine

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Manuscripts.....

Moreland, R.M., Dresser, M.E., Rodgers, J.S., Roe, B., Conaway, J.W., Conaway, R.C., and Hanas, J.S. (2000). Identification of a protein that interacts with vertebrate transcription factor IIIA. *Nucleic Acids Research* 28, 1986-1993.

Rodgers, J.S., Hocker, J.R., Hanas, R.J., Nwosu, E.C., and Hanas, J.S. (2001). Mercuric ion inhibition of eukaryotic transcription factor binding to DNA. *Biochemical Pharmacology* 61, 1543-155.

Hanas, J.S., Madhusudhan, K.T., Moreland, R.M, Hocker, J.R., Lerner, M., and Brackett, D. (2001) cDNA cloning, DNA binding, cell localization, and evolution of mammalian transcription factor IIIA. *Gene*, In review.

Trachte, A.L., Hitt, D.C., Lerner, M.R., Hanas, J.S., Jupe, E.R., Lightfoot, S., Brackett, D.J., and Postier, R.G. (2001) Adenoviral delivery of constitutively activated bone morphogenetic protein recepror 1B induces changes in phenotype and gene expression of C2C12 cells. *Molecular Therapy*, in review.

Trachte, A.L., Lerner, M.R., Hanas, J.S., Jupe, E.R., Lightfoot, S., Brackett, D.J., and Postier, R.G. (2001) DNA array analysis of five patients with pancreatic adenocarcinoma: identification of genes expressed in common. *Molecular Therapy*, in review.

Hanas, J.S. and Hocker, J.R. (2001) Selenite inhibits DNA binding mechanism of Cys_2His_2 zinc finger proteins. *Biochemical Pharmacology*, in review.

Scientific personnel....James Hocker (technician), Justin Rodgers (technician), Yong-Gang Cheng (post-doc), Jason Larabee (graduate student), Evelyn Nwosu (pharmacy student), Leslye Strasia (pharmacy student), Alicia Penn (undergraduate student), Todd Karsies (medical student).

Inventions/Patents.....U.S. Patent No. 6,235,538; serial no. 09/034,705; Test for detecting substances which alter the conformational structure of zinc fingers; Inventor, Jay S. Hanas, Ph.D.

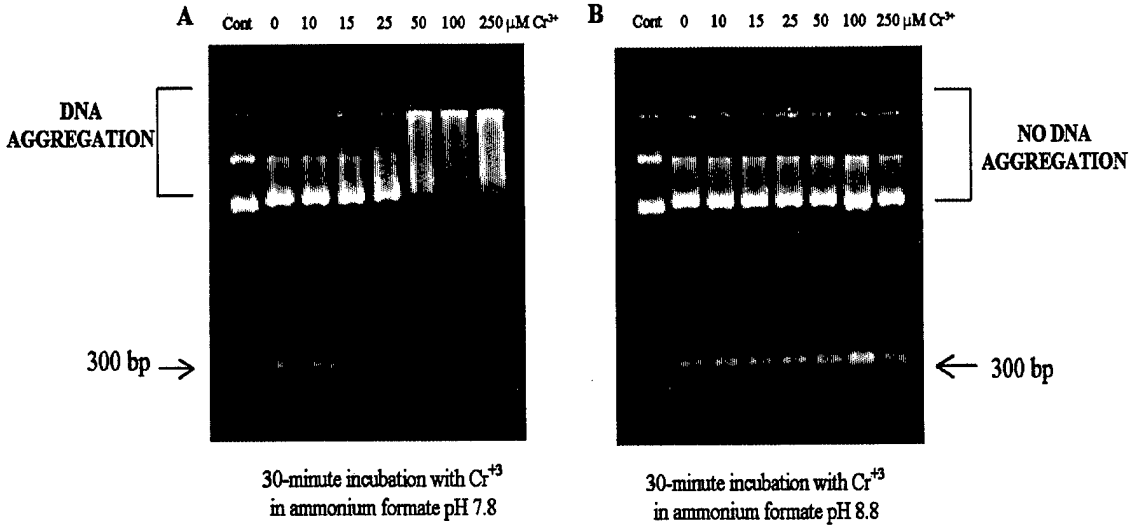
Collaborators.....Lawrence Fechter (University of Oklahoma Health Sciences Center): Dr. Fechter is an established toxicologists who will collaborate with the P.I. in animal studies involving the examination of gene expression effects of various organic solvents of interest to the military. Bruce Briggs (Wright-Patterson AFB, OH); Dr. Briggs is an established reproductive toxicologist who will collaborate with the P.I. in providing various tissues from rats exposed to JP-8 jet fuel and possibly other potential reproductive toxins. David Sonntag (Wright-Patterson AFB, OH); Maj. Sonntag is a molecular toxicologist interested in DNA damaging agents and their mechanism of action. He will collaborate with the P.I. on studies involving DNA damage induced by chromium and also will provide tissues and blood samples from animals and humans exposed to JP-8 jet fuel.

Key findings.....

1) Chromium III causes DNA aggregation:

Chromium is a common industrial toxicant that inhibits cellular proteins and binds to DNA. The study described herein was designed to begin probing the mechanism of interaction of chromium III with DNA. This information will be useful in elucidating potential disease mechanisms in humans caused by exposure to this toxicant. Chromium III was chosen for study because it is relatively safe to work with *in vitro* and is the toxic form in the cell once reduced from chromium VI which is the form of chromium that readily enters cells. Using a DNA agarose gel electrophoresis assay, we found that micromolar concentrations (25-50 μM) of chromium III caused plasmid DNA to migrate abnormally at the top of the gel (Fig. 1A). This DNA aggregation phenomenon also inhibited the restriction enzyme digestion of this DNA as evidenced by the disappearance of the 300 bp DNA band at the higher chromium III concentrations (Fig. 1A). It is noteworthy that this DNA aggregation and cleavage occurs at pH 7.8 (panel A) but not at pH 8.8 (panel B). We are presently trying to elucidate this DNA aggregation mechanism and preliminary data indicates that the aggregation involves covalent modification of the DNA by chromium.

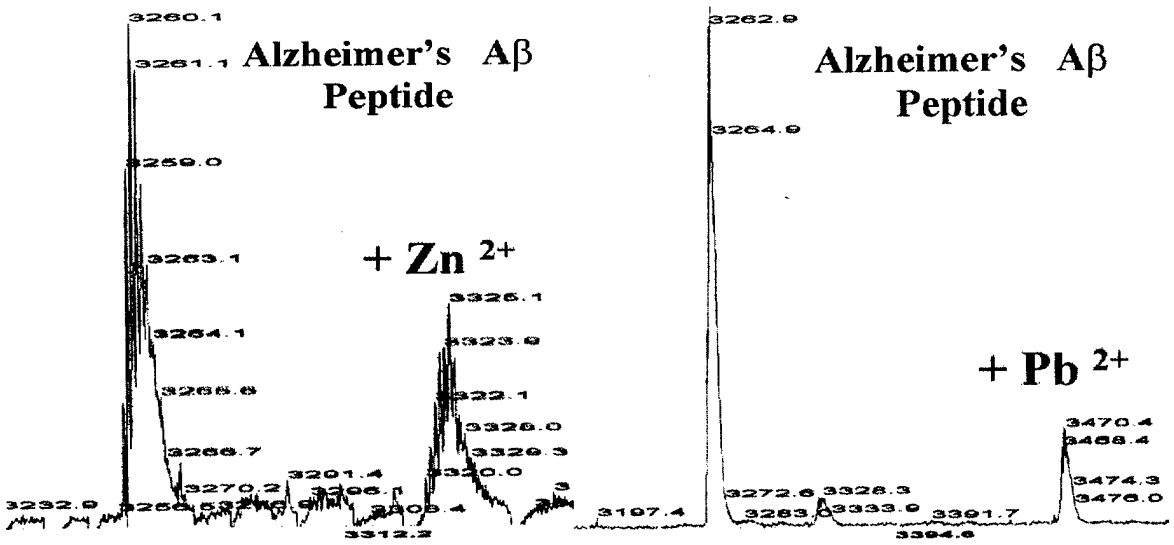
Fig. 1. Concentration and pH dependence of chromium III-induced 5S gene-plasmid DNA aggregation and restriction enzyme inhibition in formate buffer



2) Lead ions bind cellular proteins and alter gene expression:

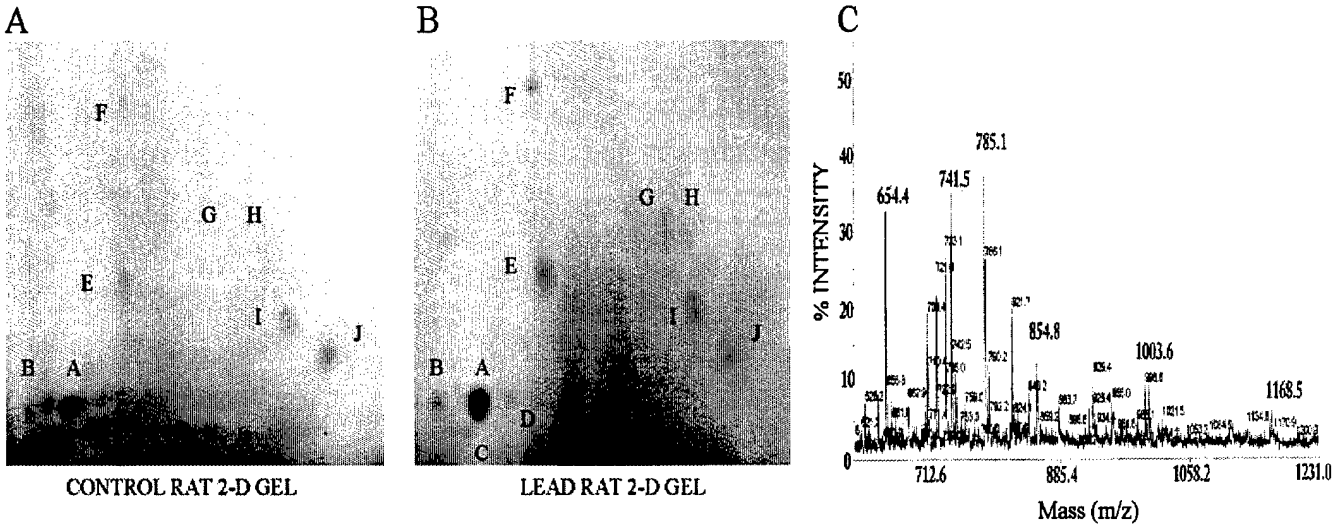
Lead is the most common metal toxicant in the environment. Lead poisoning causes many human pathologies including those of the kidney and nervous system. Our studies are focused on understanding the interactions lead ions have with various cellular constituents and also understanding any lead toxicity mechanisms involving alterations in gene expression. Understanding these mechanisms will result in better understanding of human diseases caused by lead and aid in the development of therapeutics to ameliorate such toxic effects. Fig. 2 depicts a mass spectrometric assay we have developed to look at lead binding to various cellular proteins. In this assay, the binding of a lead ion will increase the mass of the protein (Alzheimer's amyloid fragment) by a fixed amount, 207 daltons (3262.9 to 3470.4). This protein normally binds a zinc ion which only increases the mass of the polypeptide 65 datltons (3260.1 to 3325.1). This assay is capable of rapidly screening the binding of chemicals and xenobiotics to a variety of polypeptides. With DoD funds, we have recently acquired a Finnigan LCQ mass spectrometer that is capable of MS/MS structure determination of peptides. This instrument will allow us to identify the amino acids and peptides various xenobiotics interact with.

Fig. 2. Binding of zinc or lead ions to the amyloid polypeptide as assayed by electrospray-time of flight mass spectrometry



We have also discovered recently that rats exposed to lead ions have altered patterns of gene expression in the various tissues assayed. Fig. 3 exhibits a proteomic approach to understanding this phenomenon. Panels A and B exhibit 2D gel analysis of proteins from the kidney of a control rat (A) and from a rat exposed to 50 mg/kg lead ions for two weeks (B). In the lead exposed rat, a prominent protein (spot C) is missing and we would like to identify this protein which is down-regulated upon lead exposure. The identification of such proteins will provide information about the lead toxicity mechanism and also may aid in the design of therapeutics. Panel C is a mass spectrogram of the peptides generated from this protein. Accurate mass determination of these peptides followed by data base searches will help us identify this protein. For exact identification, peptide sequence information is required. We recently obtained a Finnigan LCQ ion trap mass spectrometer using DoD funds and this instrument will allow us to identify proteins from 2D gels in unequivocal fashion. We will utilize this proteomics approach to analyze protein expression changes in other toxicant-rat model system including chromium, other metals and inorganics, and organic chemicals. We are also utilizing DNA arrays to elucidate toxicant-induced changes in gene expression (see next section).

Fig. 3. Proteomic analysis of gene expression changes in lead exposed rats



3) JP-8 jet fuel down regulates gene expression in intestine of exposed rats

This fuel is now commonly used in all branches of the military. Since the inception of its use, reports of its toxicity to personnel handling this fuel have been numerous. We have undertaken studies to elucidate any changes in gene expression that are induced by this fuel in a rat model system. Such gene expression changes will provide clues to the toxicity mechanisms and will aid in the design of therapeutics to ameliorate deleterious effects of this fuel on personnel. This fuel was previously shown to result in the reduction in weight of rat pups from pregnant mothers exposed to the fuel and also in adult rats. To see whether intestinal effects of JP-8 are occurring and whether they may be responsible for animal weight loss, we performed DNA array analysis (Fig. 4) on rat intestine RNA from animals exposed for 91 days to the fuel via inhalation (1000 mg/cubic meter air; inhalation experiment performed by Dr. Bruce Briggs). The vast majority of the genes whose expression is affected are down-regulated (blue dots); up-regulated genes are in red. These genes down regulated in the intestine in response to JP-8 exposure are listed in Table 1. The majority of the genes down-regulated fall into the membrane receptor, kinase, and metabolic enzyme categories. These results indicate that JP-8 is down regulating signal transduction and metabolic pathways in affected intestines; both mechanisms could conceivably be responsible for the observed weight loss in animals. Future experiments will focus on examination of other tissues in JP-8 treated animals, analysis of other routes of exposure, performing

proteomics analysis (as depicted in Fig. 3) on these tissues, and designing therapeutics to ameliorate the effects of JP-8 in humans.

Fig. 4. DNA array analysis of intestine RNA from JP-8 exposed rat

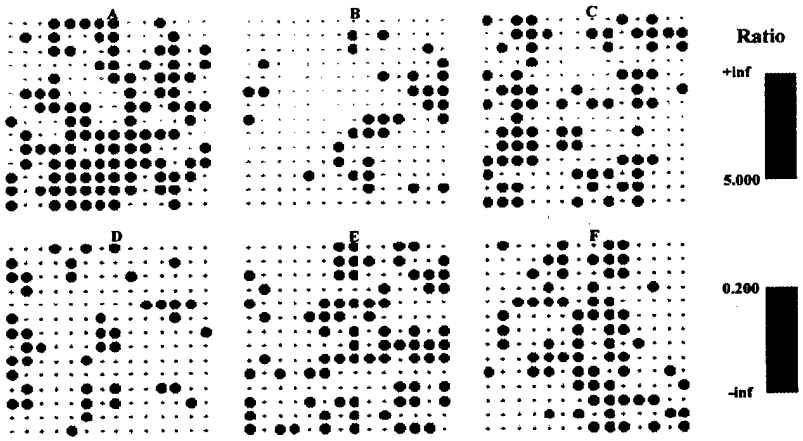


Table1. Genes down regulated in intestine by JP-8

fructose (glucose) transporter
sodium/calcium exchanger NCX2
sodium-glucose cotransporter 1
sucrase isomaltase
glucose-6-phosphate dehydrogenase
creatine kinase, ubiquitous, mitochondrial
fatty acid synthase
platelet-derived growth factor receptor, alpha
endothelin receptor ET-B
interleukin-4 receptor
angiotensin/vasopressin receptor
vitamin D3 receptor; 1,25-dihydroxyvitamin D-3 receptor
transmembrane receptor UNC5H2.
protein kinase C epsilon type
phosphorylase kinase, catalytic subunit
Rsk; ribosomal protein S6 kinase
GSK-3 alpha; glycogen synthase kinase-3 alpha;
serine/threonine kinase PCTAIRE3
Pyruvate dehydrogenase kinase kinase precursor
nuclear tyrosine phosphatase; PRL-1; affects cell growth
protein tyrosine phosphatase PTP-S

Up regulated genes in intestine

Non-processed neurexin II-beta major